DISTRIBUTION OF TWO HALOPHILIC FUNGI IN THE EGYPTIAN SOILS AND GLYCEROL ACCUMULATION

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ABSTRACT - Using the dilution plate method and on 5-25% NaCl-Czapek's agar, *Aspergillus halophilicus* and *Scopulariopsis halophilica* were encountered, but with variable numbers and frequencies from 25 samples of each of cultivated, desert and saline soils. High concentrations of NaCl (15, 20%) are convenient for isolation of these 2 species. Results reveal that there is no correlation between the distribution of these 2 halophilic species and soil textures or plant types. However, the former species was common in desert and cultivated soils, the latter prevalent in saline soils. Intra- and extracellular glycerol were markedly accumulated by *Aspergillus halophilicus* and *Scopulariopsis halophica* in response to increased salinity. Maximum accumulation was reached within the first few hours after salinization and desalinization respectively.

RÉSUMÉ - *Aspergillus halophilicus* et *Scopulariopsis halophilica* ont été isolés sur milieu Czapek-NaCl (5-25%) à partir de sols cultivés, désertiques et salins. De fortes concentrations de NaCl (15, 20%) sont favorables à l'isolement de ces 2 espèces. Les résultats montrent qu'ils n'existe pas de corrélation entre la distribution de ces 2 espèces halophiles et les types de sols (et de plantes). Cependant, la première espèce est fréquente dans les sols désertiques et cultivés, tandis que la seconde est prépondérante dans les sols salins. Du glycérol intra- et extracellulaire est accumulé par ces 2 espèces en réponse à l'augmentation de la salinité. L'accumulation maximale est atteinte dès les premières heures de salinisation ou de désalinisation du milieu.

KEY WORDS : soil fungi, halophilic fungi, *Aspergillus halophilicus*, *Scopulariopsis halophilica*, glycerol accumulation.

INTRODUCTION

Our knowledge about halophilic (or halotolerant) fungi is on the whole very limited (Bayliss Elliot, 1930; Saito, 1952; Pugh, 1962; Tresner & Hayes, 1971; Abdel-Hafez, 1981). Regarding the occurrence of *Aspergillus halophilicus (Eurotium halophilicum)* and *Scopulariopsis halophilica*, it is not clear at present whether these 2 species are of relatively rare occurrence and limited distribution,

or common in particular marine habitats. Under salt stress, the intracellular salt level is not sufficient to balance the osmolarity of the medium (Norkrans & Kylin, 1969) which is compensated by the accumulation of glycerol (Alder & al., 1982; Gadd & al, 1984). Thus, the present work was designed to study numbers, distribution and frequency of occurrences of previous species in Egyptian habitats. The accumulation of glycerol in these 2 species was also studied.

MATERIALS AND METHODS

Twenty five soil samples of each, cultivated (n° 1-25), desert (n° 26-50) and saline soils (n° 51-75) were collected under some of the dominant or cultivated plants from different places of Egypt including Nile valley, Delta area and Suez canal shore, according to the method described by Johnson & al. (1959).

The soil samples were analysed chemically for the estimation of total soluble salts, carbonates, bicarbonates, some elements (Ca^{++}, Mg^{++}, K^{+} and Na^{+}) and organic matter. A pH-meter (WGPYE model 220) was used for the determination of soil pH. Soil type was determined by the hydrometer method as described by Piper (1955).

Two halophilic fungi, namely *Aspergillus (Eurotium) halophilicus* Christensen, Papavizas & Benjamin and *Scopulariopsis halophilica* Tubaki, of the soil sample were studied using the dilution-plate method as described by Johnson & al. (1959). Twenty-five plates were used for each soil sample, 5 plates for each concentration of sodium chloride in agar medium. Modified glucose-Czapek's agar ($NaNO_3$, 3g; K_2HPO_4, 1g; $MgSO_4$. 7 H_2O, 0.5g; KCl, 0.5g; $FeSO_4$.7 H_2O, 0.01g; yeast extract, 0.5g; glucose, 10g; agar, 15g; per 1 liter) supplemented with 5, 10, 15, 20 and 25% NaCl was used as isolation medium. To these media rose bengal (1 15000) was added as a bacteriostatic agent (Smith & Dawson, 1944). Plates were incubated at 28°C for 6-8 weeks and the developing fungi were counted, identified and their numbers calculated per g dry soil.

Accumulation of glycerol: 50ml portions of liquid 1% glucose-Czapek's medium supplemented with 4, 8, 16, 20 and 24% NaCl were dispensed into each of the 250ml Erlenmeyer flaks. One ml aliquots of spore suspension of either *Aspergillus halophilicus* and *Scopulariopsis halophilica* were used as inocula and the flasks (in duplicates) were then incubated at 28°C for 20 days. Growth was measured as dry weight of mycelium. Also, fungal cells were hypertonically or hypotonically stressed by resuspending harvested cells in 20ml medium containing 24 or 4% NaCl, respectively. At the end of incubation (0 to 10h) the cells were harvested by filtration. The reaction was stopped by the addition of trichloroacetic acid. Glycerol in the cells and the external medium was assayed by the method of Lambert & Neish (1950).

RESULTS AND DISCUSSION

Water content of soils sample varied from a low value (2.4-9.9%), a moderate value (10-15.2%) and a high value (15.3-21.9%). The highest value recorded (21.9%) occurred in cultivated soil n° 23 collected from Shibin El-Kom under

Sample n°	*Aspergillus halophilicus*						*Scopulariopsis halophilica*						
	Cultivated		Desert		Saline		Cultivated		Desert		Saline		
C. D. S.	15%	20%	15%	20%	15%	20%	15%	20%	15%	20%	15%	20%	25%
1 26 51	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	5.0	0.0	0.0	0.0
2 27 52	40.0	0.0	3.3	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3 28 53	16.7	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4 29 54	26.7	0.0	26.7	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5 30 55	20.0	0.0	16.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6 31 56	13.3	0.0	13.3	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7 32 57	23.3	20.0	23.3	0.0	0.0	0.0	10.0	3.3	10.0	5.0	6.7	0.0	0.0
8 33 58	3.3	15.0	10.0	0.0	3.3	0.0	10.0	11.7	0.0	0.0	0.0	0.0	0.0
9 34 59	50.0	43.3	13.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10 35 60	3.3	11.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11 36 61	0.0	0.0	6.7	0.0	0.0	0.0	0.0	0.0	20.0	10.0	3.3	15.0	0.0
12 37 62	6.7	21.7	0.0	0.0	0.0	0.0	3.3	0.0	0.0	0.0	3.3	13.3	0.0
13 38 63	20.0	11.7	23.3	13.3	10.0	1.7	0.0	0.0	0.0	0.0	3.3	0.0	0.0
14 39 64	0.0	0.0	13.3	35.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15 40 65	0.0	0.0	0.0	6.7	16.7	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16 41 66	0.0	0.0	3.3	0.0	6.7	0.0	0.0	0.0	0.0	0.0	3.3	0.0	0.0
17 42 67	0.0	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18 43 68	0.0	0.0	3.3	3.3	0.0	0.0	0.0	0.0	0.0	0.0	40.0	8.3	0.0
19 44 69	0.0	0.0	23.3	11.7	0.0	0.0	0.0	0.0	0.0	1.7	130.0	140.0	8.3
20 45 70	0.0	0.0	10.0	5.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0	0.0
21 46 71	0.0	0.0	13.3	5.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	1.7	1.7
22 47 72	0.0	0.0	0.0	5.0	0.0	0.0	10.0	0.0	0.0	0.0	90.0	0.0	0.0
23 48 73	0.0	0.0	6.7	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24 49 74	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	135.0	3.3
25 50 75	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0	3.3	5.0	0.0
TSC	223.3	131.7	219.8	110.0	53.3	5.0	33.3	15.0	33.3	26.7	306.5	318.3	13.3
TGC	1240.0	673.3	2303.0	933.8	786.7	66.7	33.3	15.0	63.3	26.7	316.6	318.3	13.3
TC	1326.7	688.3	2460.0	560.0	1112.6	385.0	1326.7	688.3	2460.0	560.0	1112.6	385.0	30.0
NCI	11	7	17	11	7	2	4	2	3	5	11	7	3
OR	M	M	H	H	M	R	L	R	L	L	M	M	L

Table 1 - Counts (calculated per g dry soil), numbers of cases of isolation (out of 25) and occurrence remarks of 2 halophilic species recovered from 25 samples of each of cultivated, desert and saline soils on 15, 20 and 25% NaCl-Czapek's agar at 28 ± 2°C (TSC: total species count; TGC: total genus count; TC: total count of fungi; OR: occurrence remark, H: high occurrence, 13-25 cases (out of 25 samples), M: moderate occurrence, 6-12 cases, L: low occurrence, 3-5 cases, R: rare occurrence, 1 or 2 cases).

Tableau 1 - Isolements de 2 espèces halophiles à partir de sols cultivés, désertiques et salins (25 échantillons de chaque) sur milieu Czapek-NaCl (15, 20 et 25%) à 28 ± 2°C.

Vicia faba. The soil samples were generally poor in organic matter content (0.02-1.98% of dry soil) and this agrees with the results previously obtained for different types of Egyptian soils (Abdel-Hattah & al., 1977; Batanouny & Abo-Sitta, 1977; Moubascher & Abdel-Hafez, 1978).

Concerning total soluble salts content, cultivated (0.13-1.69% of dry soil) and desert (0.03-1.6%) soils were relatively poor; in saline soils total soluble salts varied widely from a moderate value (6.62-9.21%, 7 samples), to a high (11.42-14.65%, 10 samples) or a very high value (15.86-18.63%, 8 samples). Moubasher & Moustafa (1970), Moubasher & Abdel-Hafez (1978), Abdel-Hafez & al. (1978) and Abol-Nasr (1981) found that the salinity of Egyptian soils ranged widely from 0.06-38.75%. Amount of recorded carbonates, bicarbonates

and chlorides fluctuated markedly from 1.65-5.94, 0.18-1.93 and 0.07-4.14%, respectively. The amount of elements also varied widely with Ca^{++} ranging from 0.03-3.7, Mg^{++}: 0.02-1.23, K^{+}: 0.02-0.88 and Na^{+}: 0.12-39mg/g dry soil.

The pH values of cultivated and saline soils were all in the alkaline side (7.2-8.9), but in the desert soils they were around neutrality (6.9-7.4). Similar observations were obtained by Batanouny & Abo-Sita (1977) and Moubasher & Abdel-Hafez (1978). Finally, the soil type of samples were as follows: cultivated soils: 18 clay, 5 clay-loam and 2 sandy-clay; desert soils: 9 sandy, 7 sandy-clay, 4 sandy-loam and 5 sandy-clay-loam; and salt marshes soil: 6 sandy, 12 sandy-clay and 7 sandy-clay-loam.

Aspergillus halophilicus (Eurotium halophilicum) is characterized by its unusually osmophilic or halophilic nature, growing optimally on substrates containing high concentrations of sugar (sucrose) or salt (NaCl). On 15 and 20% sodium choride-Czapek's agar the mean total count of this species widely varied between 3.3-50, 3.3-26.6, and 3.3-16.7 colonies g dry soil in cultivated, desert and saline soils, respectively (Tab. 1). The highest counts in cultivated, desert and saline soils and on the 2 salt concentrations were shown by samples n° 9 (clay), 29 (sandy-clay) and 65 (sandy) collected under *Triticum durum*, *Zygophilum coccinum* and *Liminiastrum monopetalum*, respectively. These samples contained 0.62, 0.17 and 0.26% organic matter and 0.27, 0.66 and 18.05% total soluble salts, respectively. This means that the distribution and numbers of *A. halophilicus* in soils tested are influenced by their contents of organic matter and total soluble salts but soil textures and types of vegetation or cultivated plants are not affecting. On 15 and 20% NaCl-Czapek's agar, *A. halophilicus* was encountered on 44 and 28% of cultivated soils respectively, on 68 and 44% of saline soils and 28 and 8% of desert soils. On these salt concentrations, it comprised 18 and 19.6%, 9.5 and 20.6%, 6.8 and 7.5% of total *Aspergillus* observed for the soil types respectively; these percentages represents 16.8 and 19.1%, 8.9 and 19.6%, 4.8 and 1.3% of total fungi of the same soil types. *A. halophilicus* was completely absent on 5, 10% and eliminated on 25% NaCl agar plates. Tresner & Hayes (1971) found that about 70% of aspergilli could withstand 20% NaCl and nearly half survived at 25% level. Abdel-Sater (1987) rated this species as highly halophilic (best growth on 20% NaCl). Raper & Fennell (1977) in their treatise on the genus *Aspergillus*, reported that the *A. glaucus* group (to which belong *A. halophilicus)* grow better on media containing 20 to 40% sugar, or an equivalent molar concentration of NaCl. *A. halophilicus* requires substantially greater amounts of these substances for optimum growth and development.

Scopulariopsis halophilica is characterized by its unusual osmophilic nature. This species is clearly related to *S. candida* (Guéguen) Vuillemin in terms of the morphology of both its conidiophores and conidia. Conidia of the latter species are close to those of the present species in the diameter. However, the latter species is certainly non-osmophilic. On 15 and 20% NaCl agar plates the mean total counts of *S. halophilica* fluctuated between 3.3-10 and 3.3-11.6 (cultivated soils); 3.3-20 and 1.7-10 (desert soils); and 3.3-130 and 1.7-140 colonies g dry soil (saline soils) as shown in table 1. On 25% NaCl, in saline soils counts ranged between 1.7-8.3 colonies. Best counts of *S. halophilica* in the 3 soil types and on the 2 NaCl concentrations were recorded in samples n° 8 (sandy-clay), 36 (sandy) and 69 (sandy-clay) gathered under *Saccharum officinarum*, *Phragmites communis* and *Salicornia fruticosa*, respectively. These samples contained

NaCl %	*Aspergillus halophilicus*	*Scopulariopsis halophilica*
0*	-	-
4	2.17	1.35
8	2.32	2.00
12	3.21	3.53
16	4.88	4.89
20	5.81	5.06
24	3.53	4.00

*Control : no growth

Table 2 - Effect of increasing the external salinity on the dry weight of 2 halophilic fungi (mg/ml).

Tableau 2 - Effet de l'augmentation de la salinité du milieu sur le poids sec de 2 champignons halophiles (mg/ml).

15.9, 3.6 and 11.1% moisture content; 0.62, 0.4 and 0.35% organic matter; and 0.83, 0.57 and 11.83% total soluble salts, respectively. This fungus was completely absent on 5, 10 and 25% NaCl agar plates in case of cultivated and desert soils, but it only eliminated on 5 and 10% NaCl agar in case of salt marshes soil. It emerged from 16 and 8%, 12 and 20%; and 44, 28 and 12% of the samples constituting 100 and 100%; 52.6 and 100%; and 96.8, 100 and 100% of total *Scopulariopsis*; these figures represent 2.5 and 2.2%; 1.4 and 4.8%; and 27.5, 82.7 and 44.3% of gross total count of fungi on the previous NaCl concentrations from the 3 soil types, respectively. This species was isolated previously for the first time from Osaka, Japan from salted seaweed, *Undaria pinnatifida* (Tubaki, 1973).

Present results reveal that there is no correlation between the distribution of the previous 2 halophilic species and soil textures or types of plant. But *S. halophilica* was prevalent in saline soils compared with cultivated and desert ones. On the contrary, *A. halophilicus* was more common in desert and cultivated than saline soils. Also, the higher concentrations of salt (15 and 20% NaCl-Czapek's agar) proved to be very convenient for the isolation of these 2 halophilic species from various soil types. On the other hand, in numerous cases the high counts of these 2 halophilic species were observed in soil samples which contained high value of total soluble salts and vice versa.

Glycerol accumulation

Aspergillus (Eurotium) halophilicus and *Scopulariopsis halophilica* were grown for 20 days in saline media having NaCl ranging from 4 to 24%: dry weight increased with the increase of the external NaCl concentration (Tab. 2). However, the dry weight of the 2 fungi under 24% NaCl concentration was slightly decreased.

Growth of the 2 species in saline media showed an enhanced production of glycerol with the major portion of the glycerol produced retained within the

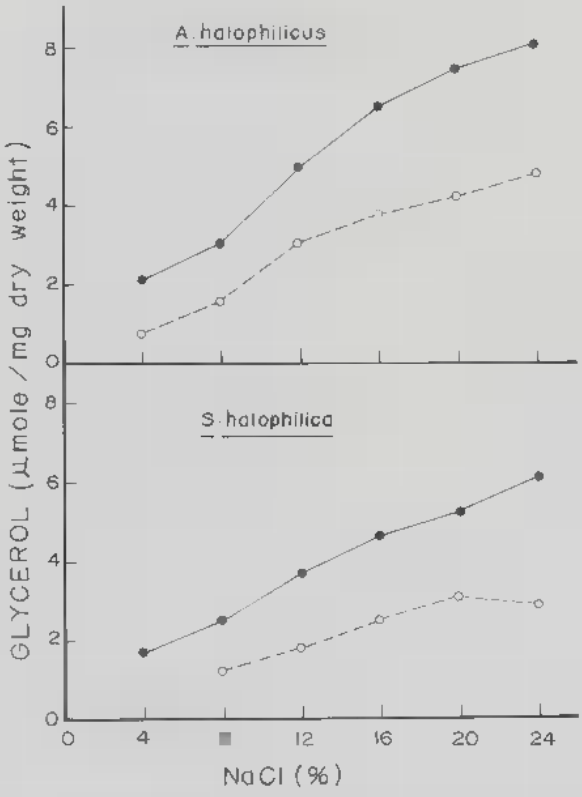

Fig. 1 - Effect of increasing the external salinity on glycerol accumulation by 2 fungi. Intracellular (■——— •) and extracellular glycerol (0- - - -0).

Fig. 1 - Effet de l'augmentation de la salinité du milieu sur l'accumulation de glycérol intra- (•———•) et extracellulaire (0- - - -0).

mycelium (Fig. 1). Glycerol has been previously reported to be accumulated in response to osmotic stress in algae (Wegmann, 1984), yeasts (Alder & Gustafsson, 1980; Alder & al., 1985) and filamentous fungi (Alder & al., 1982; Gadee & al., 1984; Zidan & Abdel-Mallek, 1987). The accumulation of internal glycerol in the first few hours after salinization (Fig. 2 A) confirms the previous findings for yeasts (Alder & al., 1985). After desalinization the internal glycerol was excreted to the external medium in the first few hours (Fig. 2 B). This is in accordance with the results obtained by Alder & al. (1982) in yeast. This means that most of the glycerol could not be internally metabolized to other organic constituents in these fungi at least under the experimental conditions used.

During salt stress, the intracellular salt level is not sufficient to balance the osmolarity of the medium (Norkrans & Kylin, 1969; Hobot & Jennings, 1981).

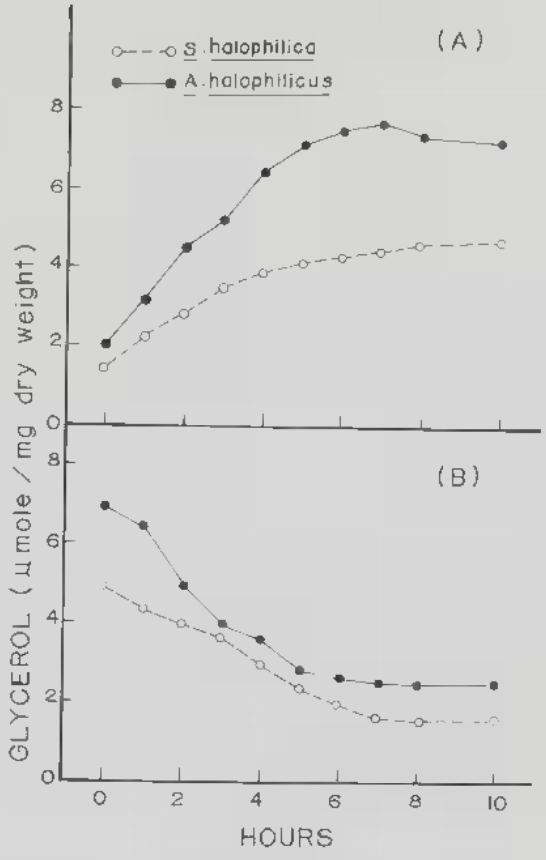

Fig. 2 - Effect of time-course on intracellular glycerol content of *A. halophilicus* and *S. halophilica*. A: the fungus cells were grown for 20 days in normal medium containing 4% NaCl and then transferred to a medium containing 24% NaCl. B: the fungus cells were grown in medium containing 24% NaCl for 20 days and then transferred to a medium containing 4% NaCl.

Fig. 2 - Dosage du glycérol intracellulaire de *A. halophilicus* et *S. halophilica*. A: croissance des champignons pendant 20j sur milieu à 4% de NaCl puis transfert sur milieu à 24% de NaCl. B: croissance des champignons sur milieu à 24% NaCl puis transfert sur milieu à 4% NaCl.

This is compensated for by the accumulation of glycerol (Gustafsson & Norkrans, 1976). The results of this investigation indicate a high degree of correlation between the internal concentration of glycerol and the external concentration of NaCl. It was suggested that, the major function of glycerol is to maintain the osmotic balance. Besides the proposed functions of glycerol as an osmoregulator and as a compatible solute (Gustafsson & Norkrans, 1976; Edg-

ley & Brown, 1983). Thus increase in intracellular glycerol could be due to the metabolic conversion of osmotically inactive storage carbohydrate to osmotically active glycerol.

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